

Effect of ascorbic acid supplementation on nitric oxide metabolites and systolic blood pressure in rats exposed to lead

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ABSTRACT

Background: Extended exposure to low levels of lead causes high blood pressure in human and laboratory animals. The mechanism is not completely recognized, but it is relatively implicated with generation of free radicals, oxidant agents such as ROS, and decrease of available nitric oxide (NO). In this study, we have demonstrated the effect of ascorbic acid as an antioxidant on nitric oxide metabolites and systolic blood pressure in rats exposed to low levels of lead.

Materials and Methods: The adult male Wistar rats weighing 200-250 g were divided into four groups: control, lead acetate (receiving 100 ppm lead acetate in drinking water), lead acetate plus ascorbic acid (receiving 100 ppm lead acetate and 1 g/l ascorbic acid in drinking water), and ascorbic acid (receiving 1 g/l ascorbic acid in drinking water) groups. The animals were anesthetized with ketamin/xylazine (50 and 7 mg/kg, respectively, ip) and systolic blood pressure was then measured from the tail of the animals by a sphygmomanometer. Nitric oxide levels in serum were measured indirectly by evaluation of its stable metabolites (total nitrite and nitrate (NO_x)).

Results: After 8 and 12 weeks, systolic blood pressure in the lead acetate group was significantly elevated compared to the control group. Ascorbic acid supplementation could prevent the systolic blood pressure rise in the lead acetate plus ascorbic acid group and there was no significant difference relative to the control group. The serum NO_x levels in lead acetate group significantly decreased in relation to the control group, but this reduction was not significantly different between the lead acetate plus ascorbic acid group and the control group.

Conclusion: Results of this study suggest that ascorbic acid as an antioxidant prevents the lead induced hypertension. This effect may be mediated by inhibition of NO_x oxidation and thereby increasing availability of NO.

KEY WORDS: Blood pressure, lead, ascorbic acid, nitric oxide

Introduction

Chronic exposure to low levels of lead causes high blood pressure in human and laboratory animals which persists after cessation of lead exposure.^[1,2] The mechanism is not completely understood, but it seems to be related to generation of free radicals, oxidant agents such as ROS, and decrease of available nitric oxide (NO). Exposure to low levels of lead causes the production of free radicals such as superoxide anion, hydrogen peroxide, oxygen, and hydrogen radicals.^[3-5] Oxidative stress plays an important role in lead-induced hypertension through inactivation of nitric oxide (NO) by ROS. Alleviation of oxidative stress using antioxidants could decrease blood pressure and increase available nitric

oxide (NO) in lead-induced hypertension.^[6,7] Ascorbic acid (vitamin C) a water soluble vitamin that is one of the important antioxidants in plasma and cell membranes that could act as a free radical (specially superoxide anion) scavenger and could increase the available nitric oxide through both protection from oxidation and increase in eNOS (endothelial nitric oxide synthase) activity.^[8,9] Ascorbic acid also is associated with a reduction in vascular sensitivity to noradrenaline and enhancement of endothelium-dependent relaxation due to increased nitric oxide bioavailability.^[10-12]

The aim of the present study was to investigate the effect of ascorbic acid supplementation as an antioxidant on blood pressure and nitric oxide metabolites level (total nitrite and

nitrate (NO_x) after acute (4-8 weeks) and subacute (12 weeks) exposure to low levels of lead acetate in rats.

Materials and Methods

Adult male Wistar rats weighing 200-250 g were divided into four groups: control (CNTL), lead acetate (Ld) (receiving 100 ppm lead acetate in drinking water), lead acetate plus ascorbic acid (Ld+AA) (receiving 100 ppm lead acetate and 1g/l ascorbic acid in drinking water), and ascorbic acid (AA) (receiving 1 g/l ascorbic acid in drinking water) groups. The animals were anaesthetized with ketamin/xylazine (50 and 7 mg/kg, respectively, ip) and then systolic blood pressure were measured from the tail of the animals by a sphygmomanometer (PE300, Narco Biosystems) after stabilization of vital signs. Nitric oxide levels in serum were measured indirectly by evaluation of its stable metabolites nitrite and nitrate by the spectrophotometric method using the Griess reaction. A good correlation between endogenous NO production and nitrite/nitrate (NO_x) levels in plasma, serum, and urine has been reported.^[13] In the present study, nitrate was reduced to nitrite by vanadium chloride(III). Diazotization of sulfanilamide by nitrous acid and its conjugation with a bicyclic amine produced a chromophore which had maximum absorbance at 540 nm.^[14,15] In addition, the weight of animals was measured in different weeks. Blood ascorbic acid concentration was measured in each group after 12 weeks using the spectrophotometric method. The serum ascorbic acid was reduced to dehydroascorbic acid by Cu^{2+} and then conjugated with the aromatic dinitro-phenyl hydrazine to produce a chromophore which had maximum absorbance at 520 nm.^[16]

Statistics

Statistical comparison of data between the experimental groups with those obtained from the control group was performed by one-way ANOVA and then the Tukey-HSD test. The repeated measurement method used for comparison of data from the same groups but in different weeks. In all cases, a value of $P < 0.05$ was considered statistically significant. Data are presented as means \pm SEM.

Results

Animal Weights

There was no difference in animal weights among different experimental groups and the CNTL before the experiment and after 4, 8 and 12 weeks of receiving lead acetate and ascorbic acid [Figure 1].

Ascorbic Acid

The serum ascorbic acid concentration in the AA group (1.23 ± 0.01 mg/dl) and Ld+AA group (1.2 ± 0.01 mg/dl) was significantly higher than that in the CNTL group (0.8 ± 0.008 mg/dl) and Ld group (0.78 ± 0.007 mg/dl) after 12 weeks [Figure 2]. There was no significant difference in the serum ascorbic acid concentration between the AA group and Ld+AA group, as well as between the CNTL group and Ld group after 12 weeks [Figure 2].

Blood Pressure

After 4 weeks of lead treatment, systolic blood pressure in the Ld group was elevated (110.28 ± 4.88 mmHg) but was not significantly different in comparison with the CNTL group

Figure 1: Animal weights in different groups. (CNTL: control group; AA: ascorbic acid group; Ld: lead acetate group; Ld+AA: lead acetate + ascorbic acid group)

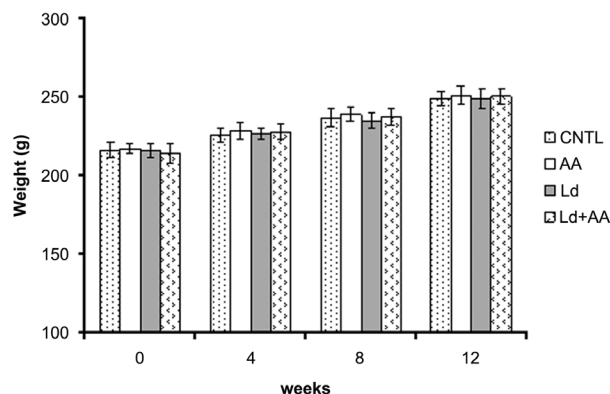
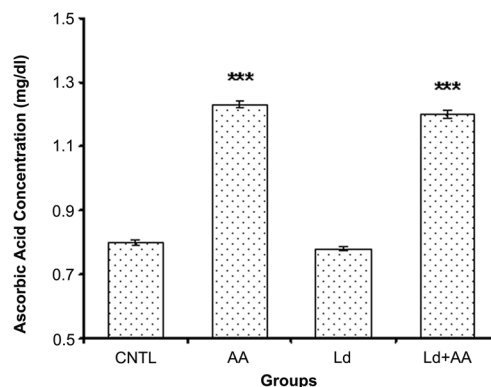


Figure 2: The serum ascorbic acid concentration on 12th week



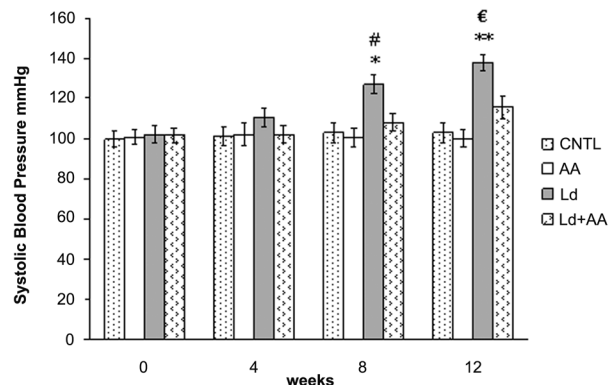
(Mean \pm SEM, N=7); *** $P < 0.001$ vs the CNTL group.

(101.28 ± 4.55 mmHg). Persistent usage of lead acetate increased the systolic blood pressure significantly to 127.14 ± 4.83 mmHg and 137.85 ± 4.14 mmHg level after 8 and 12 weeks, respectively, as compared to the value in the CNTL group [Figure 3]. No significant difference in the systolic blood pressure between the Ld+AA group and the CNTL group in 4th, 8th and 12th week was seen. There was no significant difference in the systolic blood pressure of the AA group and the CNTL group in 4th, 8th and 12th week [Figure 3].

Nitric Oxide

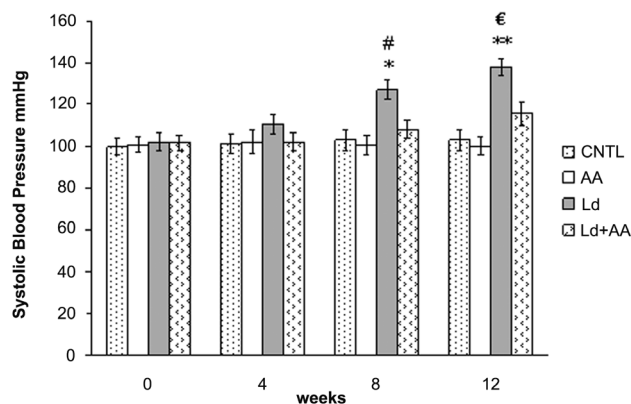
After 4 weeks, the NO_x level in the Ld group (46.05 ± 1.69 μM) significantly decreased relatively to the CNTL group (54.18 ± 1.63 μM) [Figure 4]. Prolonged administration of lead acetate ended into a more decrease in NO_x level, there was a significant decrease in NO_x level in the Ld group after 8 weeks (39.03 ± 2.10 μM vs 53.77 ± 1.59 μM) and after 12 weeks (32.12 ± 1.57 μM vs 52.06 ± 1.96 μM) as compared to their corresponding control value. NO_x level in the Ld group after 8 weeks significantly decreased relatively to NO_x level in the Ld group before the experiment. Also, there was significant decrease in NO_x level in the Ld group after 12 weeks in comparison with the NO_x level in the Ld group after 4 weeks [Figure 4]. There was no significant difference in NO_x level

Figure 3: Measurement of systolic blood pressure (Mean \pm SEM, N=7)



* $P < 0.05$; ** $P < 0.01$ vs the cont group; # $P < 0.05$ vs the Ld group in 4th week; € $P < 0.05$ vs the Ld group in 8th week

Figure 4: Measurement of NOx concentration (Mean \pm SEM, N=7)



* $P < 0.05$, ** $P < 0.01$ vs the cont group, # $P < 0.05$ vs the Ld group in week zero, € $P < 0.01$ vs the Ld group in 4th week, £ $P < 0.05$ vs the Ld+AA group in week zero, † $P < 0.01$ vs the Ld+AA group in 4th week, ‡ $P < 0.01$ vs the Ld group in 12th week

between the Ld+AA group and the CNTL group, nor between the AA group and the CNTL group in 4th, 8th, and 12th week. On the other hand, there was a significant difference in NOx level between the Ld group and the Ld+AA group after 12 weeks [Figure 4].

Discussion

The present study reveals that ascorbic acid can prevent the lead-induced hypertension via blocking NO oxidation by ROS and NO reduction. In the present study, administration of lead acetate and ascorbic acid in drinking water had no effect on food intake and water drinking. Karimi *et al.* reported similar results.^[17] After 4 weeks, the systolic blood pressure increased, however non-significantly. Systolic blood pressure elevation was significant after 8 weeks of lead exposure and remained elevated after 12 weeks. These results are in agreement with another report by Ding *et al.*^[18]

Administration of ascorbic acid prevented the lead-induced hypertension; however, it seems that this prevention was not complete, nevertheless, there was no significant difference in the systolic blood pressure between the Ld+AA group and the CNTL group. There was a significant decrease in NOx level in the Ld group relative to the CNTL group in 4th,

8th, and 12th week. Although the NOx level in the Ld+AA group had decreased in comparison with the CNTL group in 4th, 8th, and 12th week, but it was not significant and it seems that the administration of ascorbic acid could prevent the decrease in NOx level and this prevention is not complete.

In recent studies, a good correlation has been reported between the blood lead levels and the hypertension.^[11] High blood pressure has been reported in both acute and chronic exposure to lead.^[19] In many studies, chronic exposure to low levels of lead caused a permanent increase in blood pressure.^[20] Lead-induced hypertension have a close correlation with the increase in free radicals production and changes in nitric oxide metabolism.^[21,22]

It is also known that the changes in blood nitric oxide level is not solely the cause of lead-induced hypertension^[18,23] and the other factors such as changes in ACE activity, in Ang II level, EDCF endothelin, and in vascular responsiveness might be involved.^[23,24]

Conclusion

The results of this study reveal that the decrease in available NO is the main mechanism of lead-induced hypertension, and ascorbic acid supplementation prevents the lead-induced hypertension via inhibition of NO oxidation and an increase in available NO.

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